Hisayoshi NOZAKI*: The life history of Japanese *Pandorina unicocca* (Chlorophyta, Volvocales)

野崎久義*: 本邦産の *Pandorina unicocca* (緑藻・オオヒゲマワリ目) の生活史について

(Pl. I~II)

Introduction Although some Japanese strains of *Pandorina morum* Bory were described in detail in the previous papers (Nozaki & Kazaki, 1979; Nozaki, 1980), the other species of *Pandorina* have not been reported from Japan. In 1979, I had a chance to obtain Japanese specimens of *Pandorina unicocca* Rayburn et Starr from Kanagawa-Prefecture. This species was described by Rayburn and Starr (1974) by its isogamous sexual reproduction and its morphological disagreement to any other species of the genus. According to them, *Pandorina unicocca* was delimited by having cells which contained a single basal pyrenoid and by colonies which had *Eudorina*-like appearance. The colony consists of cells which were separated from one another in the gelatinous envelope. They also gave a description on both asexual and sexual reproduction observed under controlled laboratory conditions, but they did not mention on the gone colony formation in detail.

In the present study, I observed the asexual and sexual reproduction, particularly the gone colony formation, in the Japanese strains of *Pandorina unicocca* under controlled laboratory conditions.

Materials and Methods *Samples* Water samples used in this study were collected at Nobi, Miura-Peninsula, Kanagawa-Prefecture, in May, 1979.

Methods The methods used in this study are the same as those in the previous study on *Pandorina morum* (Nozaki & Kazaki, 1979).

Clonal cultures were obtained by the pipette-washing methods (Pringsheim, E. G., 1946) from the samples and were grown in a synthetic medium, which was modified from M3-medium (Rayburn & Starr, 1974). Maintenance of cultures and experimental works were carried out under controlled laboratory conditions: temperature, about 20°C and illumination, about 4000 lux; 14 hrs

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light-10 hrs dark. Mating reaction was induced by mixing of the colonies of two complementary mating types in a watch glass supported on a glass triangle in petri dishes. The heterothallic mating pair of strains observed in this study was obtained by random mixing of five strains from the samples. Reddish brown zygotes were induced to germ after the dark treatment on the agar surface during about one month. Gelatinous matrix and flagella were observed by staining with methylene blue and by using a phase contrast microscope.

Results *Asexual reproduction* Cell divisions usually occur when the size of the cell attains to about 20 μm in surface diameter. Each cell of the colony performs daughter colony formation equally, but does not divide synchronously. Some of the cells lag sometimes in one colony (Pl. I, D).

Following the cell division, the gelatinous matrix of the parental colony becomes more swollen, and a transparent membrane, which is thought to have surrounded each parental cell tightly before cell division, become swollen to be separated from the surface of the daughter cells (Pl. I, D). This membrane is spherical in shape and at last attains to about 32 μm in diameter.

Each daughter colony is formed in this transparent vesicle. Namely, usually 4 or 5 longitudinal divisions occur successively to form a 16 or 32-celled plakea and a spherical colony is formed as a result of inversion (Pl. I, E). During the inversion, each individual cell begins to project two flagella, one of which becomes much longer than the other when the new gelatinous matrix is secreted. Consequently the longer flagellum seems to penetrate the new matrix, while the shorter one is embedded in it. As a result, the daughter colony has uniflagellate cells apparently, and is formed in the transparent vesicle in the parental confluent gelatinous envelope (Fig. 4), which is measured about 300 μm in length in case of a 32-celled parental colony. Up to this stage, the stigma of the parental cell has remained to one of the cells of the plakea or daughter colony, but soon new stigmata appear from the individual cells. After the daughter colony has been released from the parental gelatinous matrix, a single basal pyrenoid is developed and the two flagella become equal in length in each individual cell. The length of the daughter colony just after the formation is about 23 μm in case of a 16-celled colony or about 26 μm in case of a 32-celled colony. It takes several hours from the first cell division to the release of the daughter colony from the parental gelatinous matrix.

Sexual reproduction The strains observed in this study are heterothallic

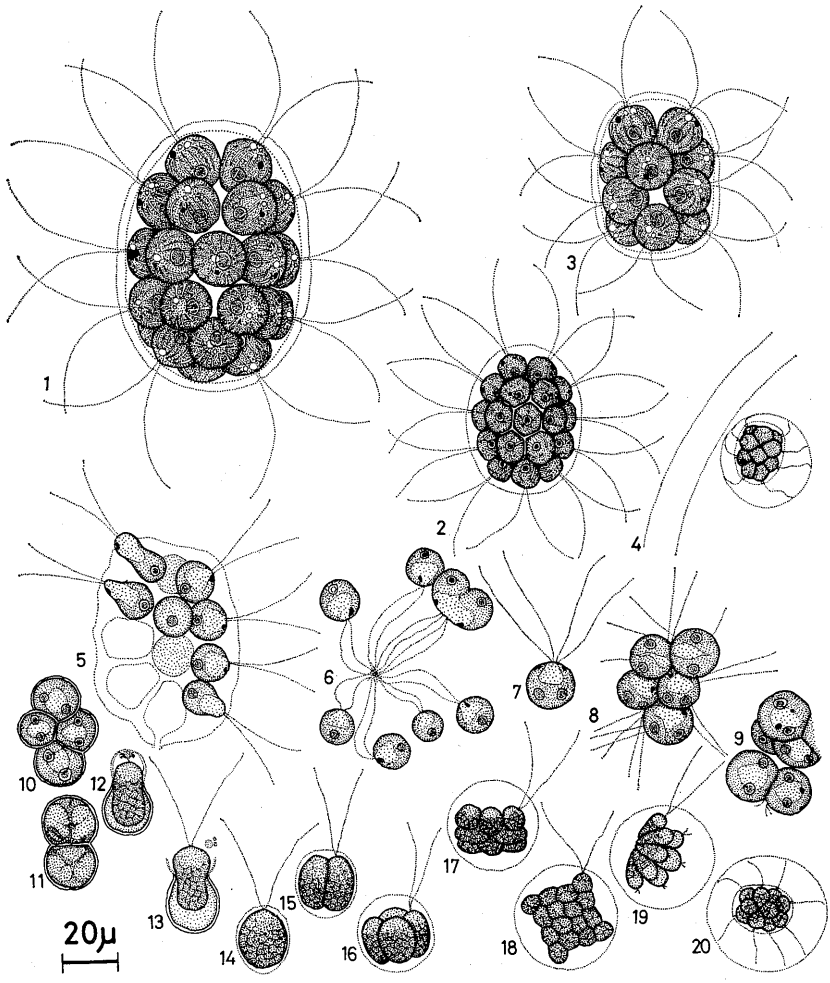

Fig.s 1–20. *Pandorina unicocca* Rayb. et Starr.
1: 32-celled matured colony. 2: 32-celled young colony. 3: 16-celled matured colony. 4: Daughter colony in transparent vesicle in confluent gelatinous envelope of parental colony. Note apparently uniflagellate cells and parental stigma in one of the cells. 5: Gamete release. 6: Gamete clumping and fusing gametes. 7: Quadriflagellate zygote. 8: Motile zygotes aggregating. 9: Aplanozygotes before secretion of wall. 10: Green zygotes with heavy wall. 11: Reddish brown zygotes. 12: Germinating zygote, protoplast projecting two flagella into space formed by thin-walled protuberance. Note hyaline bodies in this space. 13: Biflagellate gone cell escaping from zygote wall. Note three hyaline bodies. 14: Biflagellate gone cell with gelatinous envelope. 15–20: Gone colony formation; 15: 2-celled stage. 16: 4-celled stage. 17: 8-celled stage. 18: 16-celled stage. 19: Inversion stage of 16-celled plakea. 20: 16-celled gone colony in gelatinous envelope. Note apparently uniflagellate cells.

and the mating reaction occurs soon after the mixing of the colonies of two complementary mating types.

The process of the mating reaction is essentially the same as that of *Pandorina morum* (Nozaki & Kazaki, 1979). Colony clumping (Pl. I, G), gamete release (Fig. 5), gamete clumping and conjugation of gametes (Fig. 6) occur successively, and quadriflagellate zygotes are formed (Fig. 7). These motile zygotes soon begin to aggregate (Fig. 8) and enter the dormant period with their flagella disintegrated (Fig. 9). In the earliest case, the clump of these aplanozygotes is formed within one hour from the time of the mixing.

Next step, the aplanozygotes lose their stigmata, secrete heavy cell walls during the following day (Fig. 10), and then become reddish brown in colour after about one week (Fig. 11). This matured zygote measures 10–20 μm in diameter.

The zygote begins to germ within a day after transferred from the darkness to usual conditions. At the first stage, one part of the zygote wall becomes distended into a thin-walled protuberance. Then the reddish brown content (protoplast) begins to project two flagella into the space formed by the protuberance (Fig. 12). In this space, two or three hyaline bodies, which are considered to be the degenarate products of the meiotic division, are observed. Subsequent to this process, the thin-walled protuberance is ruptured and the reddish brown protoplast escapes from the wall (Fig. 13). This gone cell is somewhat ellipsoidal or oval in shape and is surrounded by a gelatinous envelope, through which the two flagella of equal length are projected (Fig. 14). This envelope is spherical in shape and becomes more swollen as the gone colony formation progresses. It measures 15 μm to 45 μm in diameter. The gone colony is formed in this transparent vesicle as it swims with these two flagella.

After swimming for several hours, the gone cell begins cell division to form a gone colony (Figs. 15-20). The process of this gone colony formation is essentially the same as of the daughter colony formation in asexual reproduction. That is, successive three, four or five divisions occur to form an 8, 16 or 32-celled plakea, and after inversion a gone colony, which has apparently uniflagellate cells and a new gelatinous matrix, is formed (Fig. 20). It takes about 5 hours from the first cell division to this stage.

The two flagella of the gone cell remains to stick to one of the daughter cells of the peripheral region of the plakea during cell divisions (Figs. 15-19).

They are however detached from the cell in the late stage of inversion and the vesicle containing the gone colony comes to a halt. The reddish brown granules recognized in the zygote still remain after the gone colony is formed, but vanish gradually after the release from the vesicle. Consequently a green colony is formed within a day. In this colony, each cell has a cup-shaped chloroplast with a single basal pyrenoid and two flagella of equal length. The size and number of cell of the gone colony depend upon the size of its former zygote. The gone colony just after the formation generally measures about 15 μm in case of an 8-celled colony and about 25 μm in case of a 32-celled colony in length.

Discussion My observation on the vegetative phase and the sexual reproduction agreed to some extent with the results reported by Rayburn and Starr (1974). But the asexual reproduction was different with regard to the parental gelatinous matrix.

In the present study, each daughter colony was formed in a transparent vesicle (Fig. 4), which is thought to have surrounded the parental cell tightly in the vegetative phase. Though Rayburn and Starr (1974) did not report such a vesicle in the asexual reproduction, I clearly observed this in the same strains they had studied (103, 104, 105 and 106). This disagreement between the two observation might be caused by the difference of the methods of observation; in the present study, the materials were stained with methylene blue and observed by using a phase contrast microscope. Such a vesicle, in which each daughter colony is formed in the parental confluent gelatinous envelope, has been reported by several authors in various species of the colonial Volvocales; i.e. Hartmann (1924) and Iyengar (1933) in *Eudorina elegans* Ehrenberg, Doraiswami (1940) in *Eudorina indica* Iyengar, Merton (1908) in *Pleodorina illinoisensis* Kofoid and Chatton (1911) in *Pleodorina californica* Shaw. But, in another species of *Pandorina, P. morum* (Pringsheim, N., 1870; Dangeard, 1900; Taft, 1941; Coleman, 1959, Nozaki, 1980.), this vesicle in the asexual reproduction has not been reported. According to my observation (1980), each daughter colony was formed in a keystone-shaped space of the parental gelatinous matrix, and was not formed in a vesicle in the confluent gelatinous envelope of the parental colony.

It is considered that this difference in the asexual reproduction between *Pandorina morum* and *P. unicocca* is derived from the structural difference of the gelatinous matrix of the colony. From my observation of these two species,

this difference was also revealed when the colonies were swollen and constitutive cells became separated from each other under unfavorable conditions. Besides, the gelatinous matrix is said to maintain the colonial arrangement of cells in *Pandorina morum* (Fulton, 1978). From this point of view, the difference of the gelatinous matrix may reflect the morphological difference between the two species (Rayburn & Starr, 1974), namely the difference of the degree of the separation of the constitutive cells of the colony.

In addition, the form of the projection of new flagella in the daughter colony formation, which has been already reported by Rayburn and Starr (1974), is the same as that in *Eudorina-Pleodorina* (Goldstein, 1964). In *Pandorina morum* (Pringsheim, N., 1870; Dangeard, 1900; Taft, 1941; Coleman, 1959; Nozaki, 1980), however, apparently uniflagellate cells of daughter colonies just after the formation have not been reported. According to my observation (1980), the two flagella of each individual cell were projected equally in asexual reproduction of *Pandorina morum*.

Based on these two characters concerned with the asexual reproduction, namely, the parental gelatinous matrix and the form of the projection of the new flagella, it may be postulated that *Pandorina unicocca* is more related to the genus *Eudorina* than *Pandorina morum*; though these two genera, *Pandorina* and *Eudorina*, have been delimited by the form of sexual reproduction (Smith, 1930; Thompson, 1954; Rayburn & Starr, 1974).

I would like to express my sincere thanks to Prof. H. Kazaki of Tokyo Metropolitan University for his suggestions and guidance and to Mr. S. Kato of the same university for collecting the materials. Thanks are also due to the members of Makino Herbarium and Department of Natural History, Faculty of Science, Tokyo Metropolitan University and also to the colleagues of Keio High School for their helpful facilities.

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Explanation of Plates I~II

Plate I. *Pandorina unicocca* Rayb. et Starr. A: Optical section of 32-celled matured colony showing pyrenoids and stigmata. B: Surface view of 32-celled matured colony. C: Optical section of 16-celled matured colony. D: 2, 4 and 8-celled plakeal stages, each plakea being surrounded by vesicle. E: 16-celled plakeal and mulberry stages. F: 32-celled young colony with apparently uniflagellate cells. G: Colony clumping. H: Gamete release. I: Gamete clumping and conjugation of gametes. J: Quadriflagellate zygote. K: Motile zygotes aggregating. L: Aplanozygotes before secretion of walls, each zygote having two pyrenoids.

Plate II. *Pandrina unicocca* Rayb. et Starr. M: Green zygotes with heavy

walls and pyrenoids. N: Reddish brown zygotes. O: Germinating zygote. Note hyaline bodies in thin-walled protuberance. P: Biflagellate gone cell escaping from zygote wall. Q: Biflagellate gone cell with gelatinous envelope. R: Empty wall and zygotes. S-X: Gone colony formation. S: 2-celled stage. T: 4-celled stage. U: 8-celled stage. V: 16-celled stage. W: Inversion stage of 16-celled plakea. X: 16-celled gone colony in gelatinous envelope.

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本邦産の *Pandorina unicocca* Rayburn et Starr（緑藻・オオヒゲマワリ目）に関する報告はいまだない。筆者は神奈川県，三浦半島の野比にある池より得たこの種の無性生殖と有性生殖の過程を培養条件下で詳細に観察する事ができた。その結果は Rayburn ら（1974）の報告と基本的には一致したが，無性生殖時の親のゼラチン様膜に関して，彼らが指摘していない形質が，今回，彼らが用いた株を含めて認められた。この形質は同属の *Pandorina morum* Bory とは異なり，それが両種の形態的差異（群体を構成する細胞の密着の程度）に関係するものと思われる。

Pandorina unicocca の新鞭毛の突出の様式と，今回認められた親のゼラチン様膜に関する形質は共に *Pandorina morum* のものとは異なるが *Eudorina-Pleodorina* 属に認められる形質である。これらの事実から *Pandorina unicocca* は，*Pandorina morum* よりも *Eudorina* 属に近縁のものと推測される。

□伊沢凡人：**原色版日本薬用植物事典**（B. Izawa: Illustrated Cyclopedia of medicinal Plants (Materia Medica) of Japan) pp. 331, pls 142. 1980. 誠文堂新光社. ¥20,000. かつて 4 巻に分けて出版されたものを，今回改めて 1 巻としてまとめ，他日スライドと文献を整理して全 2 巻として上梓するという。461 種の薬用植物を科ごとにまとめ，科名のアイウエオ順に配列し，各種に図版を添えて，生育地，性状，花，漢名，生薬名，薬用部，成分，薬理作用，漢方，西洋医学，民間療法，食用，其他にわけて記述している。ことに漢方と民間療法のところに力が入っていて詳細を極めるが，これらはできるかぎり発効について要をえたものに限ってあるという。図版にも意を用いて，薬用部分を主にしてあるので参考となる。早く第 2 巻のでることを望んでやまない。

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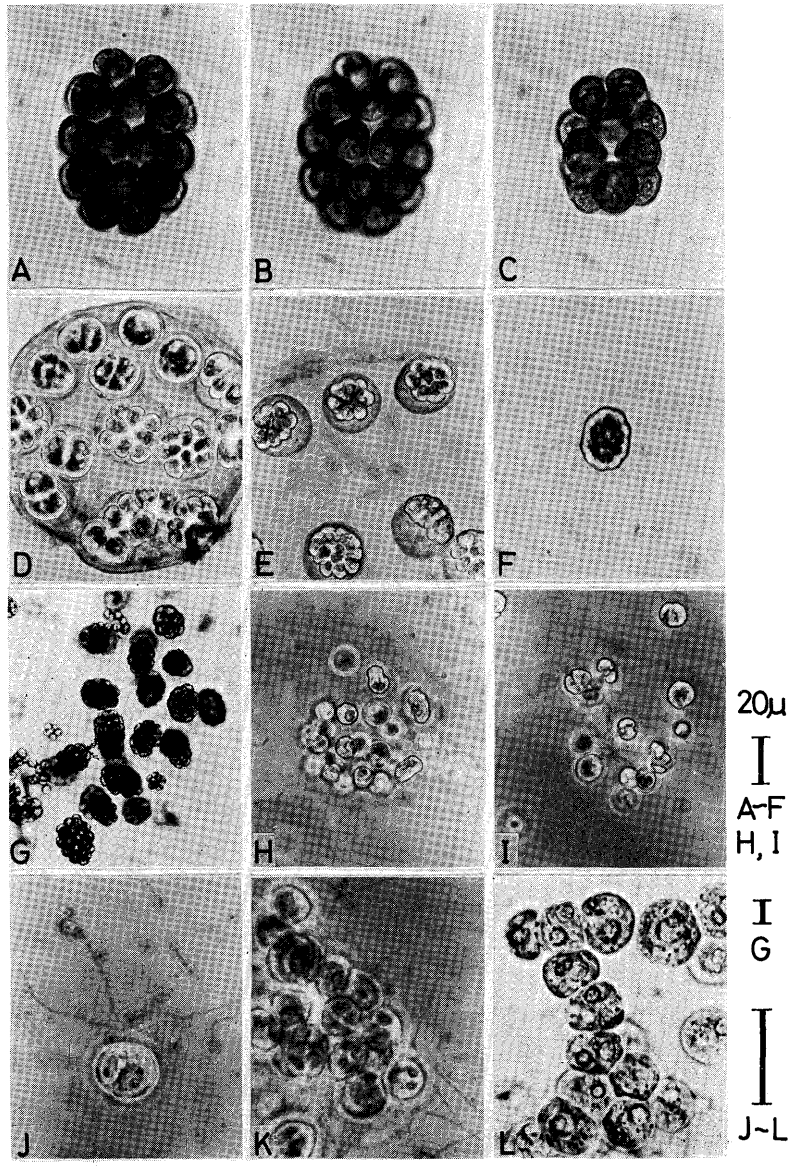
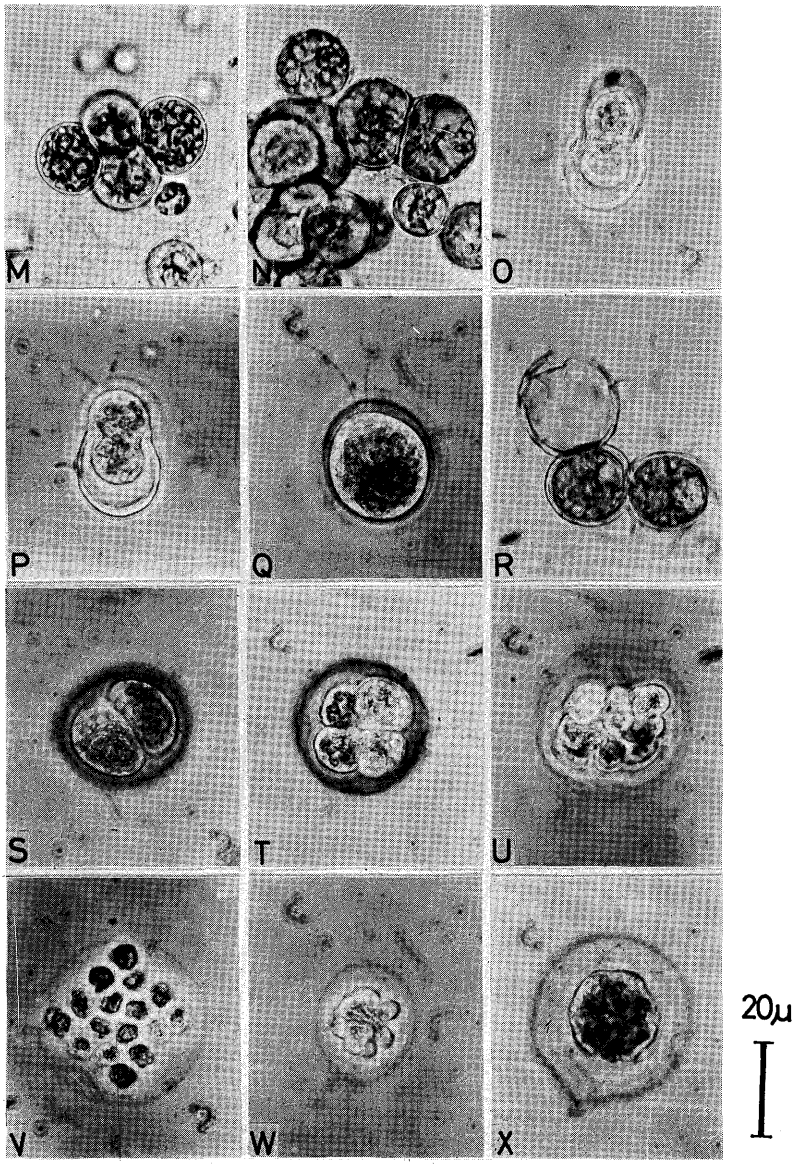


H. NOZAKI: Life history of Japanese *Pandorina unicocca*



H. NOZAKI: Life history of Japanese *Pandorina unicocca*